

FEDERAL BUREAU OF INVESTIGATION

Precedence: ROUTINE

Date: 09/11/2006

To: Washington Field
Counterterrorism

Attn: AMERITHRAX-1
Attn: WMDOU

From: Washington Field

AMX-2 / NVRA

Contact: SA [REDACTED]

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Approved By: [REDACTED]

Drafted By: [REDACTED]

Case ID #: 279A-WF-222936-SCI18 (Pending)

Title: AMERITHRAX;
MAJOR CASE 184

Synopsis: To summarize the association of ten *Bacillus anthracis* samples acquired during searches conducted at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and Battelle with RMR-1029.

Details: Phenotypic Analysis of the *Bacillus anthracis* (Ba) spore powders used in the anthrax letters sent to Senators Daschle, Leahy, and the New York Post determined that multiple morphological variants were present. When grown on solid media, the phenotypes (i.e., appearances) of these variants differ from each other, and from the ancestral Ames strain; demonstrating differences in textures, colors, and growth patterns than colonies produced by the 1981 ancestral isolate of Ames. The five variants identified were designated as morphs A, B, C, D, and E.

Analysis of the deoxyribonucleic acid (DNA) sequences from isolates of the five morphological variants led to the discovery of 28 unique genetic mutations within these five classes of morphological variants. Characterization of the DNA sequences of three Morph A isolates from the Leahy, Post, and Daschle letters revealed that these three isolates each have a different mutation in the same region of their genomes. The Morph A mutations from the Leahy, Post, and Daschle letters were named A1, A2, and A3, respectively. Molecular assays with the ability to detect trace levels of the A1 and A3 mutations in a background of predominantly wild type Ba have been developed and validated by Commonwealth Biotechnologies, Inc. (CBI).

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Similarly, characterization of the Morph D isolate from the Leahy letter revealed the presence of a 258 base pair deletion, designated the "D Deletion." Molecular assays with the ability to detect trace levels of the D deletion in a background of predominantly wild type *Ba* have been developed and validated by the Illinois Institute of Technology Research Institute (IITRI) and Midwest Research Institute (MRI).

Sixteen laboratories in the United States and laboratories in three foreign countries were determined to possess stocks of the Ames strain of *Ba* before the anthrax mailings. The FBI collected a total of 1,056 Ames isolates from these laboratories and stored them in an FBI *Bacillus anthracis* Repository (FBIR). All samples submitted to the FBIR have been analyzed for the presence of A1, A3, and D mutations using the above described assays. Only ten samples in the FBIR have all three mutations present and are listed below.

FBIR Number	Origin Laboratory	Location Acquired	Sample Identifier or Label
005-016	Battelle	JM-1 Rm 164	0114-S
044-040	USAMRIID	B3 Cold Room	
049-004	USAMRIID	B3 Cold Room	RMR-1029
049-006	USAMRIID	B3 Cold Room	
049-008	USAMRIID	B3 Cold Room	
049-016	USAMRIID	Bldg. 1412, 1st Floor Cold Room	Ames Spores 2433 CDC7738
052-026	USAMRIID	B3, Rm 304	Dugway Ames spores; Dugway on the cap
053-070	USAMRIID	Bldg. 1412, Rm 212	Dugway Ames spores; $1 \times 10^{10}/\text{mL}$
054-076	USAMRIID	AA3 Cold Room	50mL tube of <i>B. anthracis</i> Ames; $3 \times 10^{10}/\text{mL}$
066-044	USAMRIID	February 2002 shipment from Ivins to Northern Arizona University	Ames strain RMR 1029 from Dugway 1997

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RMR-1029 was a large *Ba* Ames spore batch produced to conduct numerous anthrax aerosol challenges. Upon its assembly, the concentration of RMR-1029 was approximately 3.6×10^{10} /ml, consisting of one liter in total volume, split between two one liter flasks (0.5 liter each). In order to produce the quantity of spores necessary to make RMR-1029, Dugway Proving Ground was contracted to produce *Ba* Ames spores, which were combined with spores produced in-house by Bruce Ivins at USAMRIID. RMR-1029 consisted of a combination of 34 spore production dates, 22 production dates at USAMRIID and 12 production dates at Dugway, totaling approximately 3.6×10^{13} total spores, 85% of which were produced at Dugway. Due to the quantity of spores comprising RMR-1029 and the fact that this sample was one of the first to be identified as having all three morphs present, the AMERITHRAX Task Force set out to determine if, and how, the other triply positive samples were derived from, or otherwise related, to RMR-1029. The results of this investigation is described below.

FBIR Sample 005-016

On June 19, 2001, Battelle received 30 mL of *B. anthracis* Ames spores, with a Colony Forming Units per milliliter (CFU/ml) of 3.9×10^{10} /ml or 1.17×10^{12} total spores, from USAMRIID. A Battelle Material ID# of "0114-S" was assigned to the material. Slants of 0114-S submitted to the FBI Repository were generated on 4/01/2002 (Reference 279A-WF-222936-BATTELLE Serial 91). These slants were processed into the FBIR and were given FBIR sample number 005-016.

Bruce Ivins recalls sending a subsample of RMR-1029 to [redacted] at Battelle for aerosol challenges (Reference 279A-WF-222936-USAMRIID Serial 935). According to the RMR-1029 inventory log maintained for RMR-1029 Ivins disbursed 50 ml of spores on May 1, 2001 and 30 ml of spores on June 15, 2001 to [redacted] Battelle (Reference 279A-WF-222936 Serial 6263).

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FBIR Sample 044-040/049-006

This sample was identified by the FBI during the December 2003 consensual search of USAMRIID and was found in the box identified by Bruce Ivins as belonging to [redacted]. This sample entered into the FBIR on two separate occasions as FBIR numbers 044-040 (submitted to the repository by Bruce Ivins) and 049-006 (entered into the repository by NMRC after the sample was seized). [redacted] (Reference 279A-WF-222936-USAMRIID Serial 1004) identified the vial labeled "*Ames* Spores 2.3×10^{10} /ml" as

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belonging to her based on the handwriting on the vial. [redacted]
did not recall the circumstances surrounding the vial.

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FBIR samples 044-040/049-006 is likely a sub-sample of RMR-1029 based on the fact that the concentration of the sample is the same as that determined for RMR-1029 on March 16, 1999. Given that [redacted] doesn't remember the circumstances surrounding this sample, two possibilities as to the ownership of this sample arise. It is possible that this sample was used by [redacted] in her own research or the sample was prepared by [redacted] and was provided to [redacted] by [redacted] or Ivins. If the latter is the case, this sample could be the original stock of Ames spores [redacted] received from Ivins on March 24, 1999, (see discussion below of FBIR sample 049-008) which would be the most reasonable explanation for finding both samples (FBIR 049-006 and 049-008) in the box identified as belonging to [redacted]. Additionally, both [redacted] and [redacted] worked for [redacted] and after [redacted] completed his Postdoc at USAMRIID he showed [redacted] where he [redacted] stored his samples in case [redacted] needed them for future experiments (Reference 279A-WF-222936-POI Serial 1437). [redacted] consolidated [redacted] samples into one box, wrote his name on it and taped it shut. This box was moved into suite B4 after [redacted] group moved there (Reference 279A-WF-222936-USAMRIID Serial 912).

FBIR Sample 049-004

This sample was identified by the FBI during the December 2003 consensual search of USAMRIID. From a walk-in cold room (Room B311) within containment suite B3, Ivins disclosed a one liter flask labeled 7737, RMR-1029 (Reference 279A-WF-222936-USAMRIID Serial 471). This is thought to be one of the two flasks in which RMR-1029 was stored. This sample was subsequently processed into the FBIR and given FBIR sample number 049-004. [Note: When RMR-1029 was submitted to the repository by Bruce Ivins it was negative for all three morphs. See discussion below for FBIR sample 066-044.]

FBIR Sample 049-008

This sample was identified by the FBI during the December 2003 consensual search of USAMRIID (Reference 279A-WF-222936-USAMRIID Serial 471) and was found in a box, which was described as belonging to [redacted] (Reference 279A-WF-222936-USAMRIID Serial 849), by Bruce Ivins. [redacted] (Reference 279A-WF-222936-USAMRIID Serial 1489) identified the vial labeled "Ames Stock 2x10⁸ No Phenol 15July99" as

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belonging to him based on the handwriting on the vial. [redacted] indicated that he obtained his spores from Bruce Ivins and provided a copy of a document dated March 24, 1999 showing that he obtained "Ames spores - From Dr. Ivins, @ 2.3×10^{10} /ml in 1% phenol. Stored @4°C." A copy of [redacted] laboratory notebook dated July 15, 1999 revealed that [redacted] made dilutions of an Ames spore stock with an initial concentration of 2.3×10^{10} /ml to a concentrations of 2×10^8 /ml then washed the spores three times with water. [redacted] indicated that he typically washed and diluted the spores received from Ivins to remove the phenol to provide a working stock.

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On March 16, 1999, Bruce Ivins conducted an experiment to determine the CFU/ml of RMR-1029 as recorded on page 91 of his notebook (Reference Ivins' notebook number 4010). In this entry Ivins indicated that the CFU/ml originally determined for RMR-1029 was 3.6×10^{10} as determined on October 22, 1997. Ivins determined the CFU/ml of RMR-1029 on March 16, 1999 to be $2.3 - 2.4 \times 10^{10}$.

According to the inventory log maintained for RMR-1029 Ivins disbursed 1 ml of spores on March 23, 1999 [Note: the inventory does not indicated to whom the spores were given] (Reference 279A-WF-222936 Serial 6263).

FBIR Sample 049-016

On September 8, 2004 Bruce Ivins was shown a photograph of a *Bacillus anthracis* Ames spore sample in a 50ml conical tube labeled "Ames spores 2433 CDC 7738." Ivins indicated that this was a sample that he provided to [redacted] and that it was either a subsample of RMR-1029, or material that [redacted] or [redacted] had made for [redacted] (Reference 279A-WF-222936-USAMRIID Serial 935). A review of Ivins notebooks revealed that he had transferred subsamples of RMR-1029 to [redacted] on six different occasions, the first documented transfer occurred on October 4, 2001 (Reference 279A-WF-222936-USAMRIID Serial 795). The October 4, 2001 date for the initial transfer of RMR-1029 to [redacted] is in agreement with the inventory log maintained for RMR-1029 which indicates that 10ml of RMR-1029 was disbursed to [redacted] on that date (Reference 279A-WF-222936 Serial 6263).

FBIR Sample 052-026

FBIR Sample 052-026 was seized from USAMRIID Building 1425 containment suite B3 Room 304 during a consent search of USAMRIID in July of 2004. [redacted] was shown photographs of this

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sample with "Dugway Ames Spores" written on the cap of the tube and indicated that the sample and writing on the tube "could be hers" (Reference 279A-WF-222936-USAMRIID Serial 1461). [redacted] stated that she probably received the sample in the late 1990's and that she obtained her *Bacillus anthracis* Ames samples from Bruce Ivins or [redacted] could not recall why she received this sample and indicated that she did not typically share samples with other researchers.

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According to the inventory log maintained for RMR-1029 Ivins disbursed 1 ml of RMR-1029 to [redacted] on November 14, 2001 (Reference 279A-WF-222936-GJ Serial 1290). The phrase "Dugway Ames Spores" was commonly used by Ivins to describe RMR-1029.

FBIR Sample 053-070

Teresa Abshire was shown two photos of FBIR Sample 053-070 seized from USAMRIID Building 1412, room 212 by the FBI during a consent search of USAMRIID during July of 2004. These photos depict a 1.25 mL vial labeled "Dugway Ames Spores," and a 50 mL conical tube inside which the vial was found. Abshire indicated that she received this sample sometime in 2003 or 2004 from Bruce Ivins. Abshire believes that she requested the sample labeled "Dugway Ames Spores" from Ivins to see if these spores looked like the spores from the anthrax attack letters from the Fall of 2001 (Reference 279A-WF-222936-USAMRIID Serial 1418).

FBIR Sample 054-076

FBIR Sample 054-076 was seized from USAMRIID Building 1425 containment suite AA3 Room AA314 during a consent search of USAMRIID during July of 2004. [redacted] Principal Investigator, Diagnostic Systems Division (DSD), was shown a photo of sample 054-076. [redacted] indicated that she received this sample from Ivins and that until it was seized in 2004, this sample was considered DSD's *Bacillus anthracis* sample (Reference 279A-WF-222936-USAMRIID Serial 1472). [redacted] provided agents with a copy of the "Receipt for Transfer of *B. anthracis* spores," for this sample. The "Receipt for Transfer of *B. anthracis* spores," indicates that 1ml of *B. anthracis* spores (Ames Strain) at a concentration of 3×10^{10} /ml was provided to DSD on April 22, 2002 (Reference 279A-WF-222936-USAMRIID Serial 1472). The concentration of 3×10^{10} /ml is identical to the concentration of RMR-1029 as it was originally determined and described on the RMR-1029 inventory (Reference 279A-WF-222936-GJ Serial 1290 and Ivins' notebook number 4010 page 68).

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FBIR Sample 066-044

In February of 2002 (prior to the establishment of the FBIR), Bruce Ivins submitted four Tryptic Soy Agar (TSA) slants of his *Bacillus anthracis* directly to Dr. Paul Keim of the Northern Arizona University (NAU) in Flagstaff, Arizona for genetic typing. All of Ivins' samples were analyzed using Dr. Keim's Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA) and determined to be the Ames strain of *Bacillus anthracis*. Since Dr. Keim requires only a minimal amount of sample for testing, he retains the majority of each sample following analysis. One of the samples Ivins submitted to Dr. Keim was labeled, "Ames strain RMR 1029 from Dugway (1997)."

Two months later, in April of 2002, Ivins submitted four samples of *Bacillus anthracis* to the FBIR. Of particular interest, FBIR sample FBIR006-002, labeled, "Dugway Ames spores - 1997" is believed to be RMR-1029, although its label is somewhat ambiguous. Genetic testing of FBIR006-002 generated **negative** results for the A1, A3, and D mutations, further bolstering the ambiguity of this sample.

As explained above, RMR-1029 was seized by the FBI in April of 2004 and transported to NMRC. In June of 2004, NMRC submitted RMR-1029 to the FBIR, which was accessioned as FBIR049-004. The genetic testing for sample FBIR049-004 resulted in **positive** results for the A1, A3, and D mutations.

In February of 2006, the AMERITHRAX Task Force requested Dr. Keim submit to the FBIR, the samples that Ivins had sent to him in February of 2002. The samples were processed into the repository under FBIR accession number FBIR066-044. More than four years after Ivins submitted the sample to NAU, genetic testing of FBIR066-044 (labeled "Ames strain RMR 1029 from Dugway (1997)") generated **positive** results for the A1, A3, and D mutations.

Summary

RMR-1029 was submitted to the repository as FBIR samples 049-004 and 066-044 as described above. Based on the information provided above, it was determined that samples 005-016, 049-008, 049-016, and 052-026 are subsamples of RMR-1029. FBIR samples 044-040/049-006 and 054-076 are very likely subsamples of RMR-1029 based on the fact that the concentrations of these samples are identical to that described for RMR-1029. FBIR sample 053-070 is likely a subsample of RMR-1029 based on the labeling on

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the vial; additionally, this sample was received by it's
custodian from Bruce Ivins **after** the anthrax mailings in 2001.

Set Lead 1: (Info)

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AT WMDOU

Review the provided information.

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